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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.

40174

Total Pages

38

First Named Inventor or Application Identifier

Mathew J. During

Express Mail Label No.

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

ADDRESS TO:

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1. ☒ **Fee Transmittal Form**
(Submit an original, and a duplicate for fee processing)
2. ☒ **Specification** [Total Pages **20**]
(preferred arrangement set forth below)
- Descriptive title of the Invention
 - Cross References to Related Applications
 - Statement Regarding Fed sponsored R & D
 - Reference to Microfiche Appendix
 - Background of the Invention
 - Brief Summary of the Invention
 - Brief Description of the Drawings (if filed)
 - Detailed Description
 - Claim(s)
 - Abstract of the Disclosure
3. ☒ **Drawing(s)** (35 USC 113) [Total Sheets **2**]
4. **Oath or Declaration** [Total Pages **3**]
- a. ☐ Newly executed (original or copy)
- b. ☒ Copy from a prior application (37 CFR 1.63(d))
(for continuation/divisional with Box 17 completed)
[Note Box 5 below]
- i. ☐ **DELETION OF INVENTOR(S)**
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
5. ☒ **Incorporation By Reference** (useable if Box 4b is checked)
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Microfiche Computer Program (Appendix)
7. **Nucleotide and/or Amino Acid Sequence Submission**
(if applicable, all necessary)
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ACCOMPANYING APPLICATION PARTS

8. ☒ **Assignment Papers** (cover sheet & document(s)) (Copy)
9. ☐ **37 CFR 3.73(b) Statement** (when there is an assignee) ☐ **Power of Attorney**
10. ☐ **English Translation Document** (if applicable)
11. **Information Disclosure Statement (IDS)/PTO-1449** ☐ **Copies of IDS Citations**
12. ☐ **Preliminary Amendment**
13. ☒ **Return Receipt Postcard (MPEP 503)**
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14. **Small Entity Statement(s)** ☒ **Statement filed in prior application, Status still proper and desired (Copy)**
15. ☐ **Certified Copy of Priority Document(s)**
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16. ☐ **Other:**

17. If a **CONTINUING APPLICATION**, check appropriate box and supply the requisite information:☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP)of prior application No: **08/472,755**

18. CORRESPONDENCE ADDRESS

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[Page 1 of 2]

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FEE TRANSMITTAL FORM

Attorney Docket No.: **40174**

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Transmitted herewith for filing is the patent application of

Inventor: **Mathew J. During**

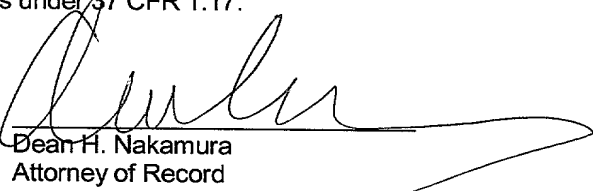
For: **ORAL DELIVERY OF ADENO-ASSOCIATED VIRAL VECTORS**

The filing fee has been calculated as follows:

(Col. 1)		(Col. 2)		SMALL ENTITY		OR OR	OTHER THAN A SMALL ENTITY	
FOR:	NO. FILED		NO. EXTRA	RATE	FEE		RATE	FEE
BASIC FEE					\$345.00			\$690.00
TOTAL	12	- 20=	0	x 9 =	\$		x 18 =	\$
INDEP	1	- 3=	0	x 39 =	\$		x 78 =	\$
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIMS				+ 130 =	\$		+ 260 =	\$
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- ☐ This application is being filed without oath or declaration and without the filing fee pursuant to 37 C.F.R. §1.53(f).
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- ☒ Any additional excess claim fees under 37 CFR 1.16.
- ☒ Any additional patent application processing fees under 37 CFR 1.17.

Dated: 27 April 2000


Dean H. Nakamura
Attorney of Record
Reg. No. 33,981

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1300 19th Street, N.W., Suite 600
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(202) 659-9076

PATENT

SMTX-006/00US

Applicant or Patentee: Mathew John During
Serial No. Or Patent No.: 08/472,755
Filed or Issued: June 7, 1995
For: ORAL DELIVERY OF ADENO-ASSOCIATED VIRAL
VECTORS

**VERIFIED STATEMENT/DECLARATION CLAIMING SMALL ENTITY STATUS
37 CFR SECTIONS 1.9(f) & 1.27(c) - NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: Yale University
ADDRESS OF ORGANIZATION: 451 College St.
New Haven, CT 06520

TYPE OF ORGANIZATION:

- ☒ University or other institution of higher education
☐ Tax exempt under Internal Revenue Service Code [26 USC 501(a) and 501(c)(3)]
☐ Nonprofit scientific or educational under statute of state or the United States of America

(Name of state:)
(Citation of statute:)

- ☐ Would qualify as tax exempt under Internal Revenue Service Code [26 USC 501(a) and 501(c)(3)] if located in the United States of America
☐ Would qualify as nonprofit scientific organization under statute of state of the United States of America if located in the United States of America

(Name of state:)
(Citation of statute:)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 19(e) for purposes of paying reduced fees under section 41(a) or (b) of Title 35, United States Code with regard to the invention identified above and described in

- ☐ the specification filed herewith.
☒ application serial no. 08/472,755 filed June 7, 1995.

☐ _____ issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

Name: Somatix Therapy Corporation
Address: 850 Marina Village Parkway
California 94501

☐ INDIVIDUAL
☒ SMALL BUSINESS CONCERN
☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING

Robert K. Bickerton

TITLE OF PERSON OTHER THAN OWNER

Director, Office of Cooperative Research

ADDRESS OF PERSON SIGNING

Yale University, New Haven, CT 06520

R. Bickerton
Signature

10/3/95
Date

PATENT

SMTX-006/00US

Applicant or Patentee: Mathew John During
Serial No. Or Patent No.: 08/472,755
Filed or Issued: June 7, 1995
For: ORAL DELIVERY OF ADENO-ASSOCIATED VIRAL
VECTORS

**VERIFIED STATEMENT/DECLARATION CLAIMING SMALL ENTITY STATUS
37 CFR SECTIONS 1.9(f) & 1.27(c) - SMALL BUSINESS CONCERN**

I hereby declare that I am:

☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to act on behalf of the concern
identified below:

NAME OF CONCERN: Somatix Therapy Corporation
ADDRESS OF CONCERN: 850 Marina Village Parkway
California 94501

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention identified above and described in

☐ the specification filed herewith
☒ application serial no. 08/472,755, filed June 7, 1995.
☐ Patent No. _____, issued _____.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify

as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

Name: Yale University
Address: 451 College St.
New Haven, CT 06520

☐ INDIVIDUAL
☐ SMALL BUSINESS CONCERN
☒ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements are made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: _____

Edward O. Lanphier II

TITLE OF PERSON OTHER THAN OWNER: Executive Vice President

ADDRESS OF PERSON SIGNING: 950 Marina Village Pkwy, #100, Alameda, CA 94501

See Above

Signature

8/18/95

Date

PATENT**ORAL DELIVERY OF ADENO-ASSOCIATED VIRAL VECTORS****ACKNOWLEDGEMENTS**

This invention was supported in part by NIH grants NS28227 and NS06208. The U.S. Government has rights in this invention as a result of this support.

INTRODUCTION**Technical Field**

This invention is in the field of gene expression and is particularly directed to expression of gene products in the gut of an animal.

Background

Adeno-associated virus (AAV) vectors have been proposed and patented as vectors for expressing gene products in animals. See, for example, U.S. patent No. 5,193,941, issued 18 August 1992, WO 9413788, as well as U.S. serial Nos. 08/227,319, the last application arising from the laboratory of the present inventor. A number of patents and other publications describe numerous AAV vectors and their uses, the uses generally being related to expression of gene products either in vitro (usually tissue cultures) or in vivo (usually in the lungs or oral mucosa, the normal sites of AAV infection, although U.S. application serial No. 08/227,319 relates to expression in the central nervous system).

Investigations in the laboratory of the present inventor have surprisingly discovered that AAV vectors can act as effective, long-term expression systems in the gut of animals after oral ingestion. This discovery provides a new method of expressing desirable gene products and control elements in the gut of animals, including humans.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the invention to provide new uses for AAV vectors that have already been developed for other purposes.

1 It is a further object of the invention to provide new recombinant AAV
2 vectors containing gut-directed gene expression systems.

3 These and other objects of the invention have been accomplished by
4 providing a method of expressing a gene product in the gut of an animal, which
5 comprises administering a recombinant AAV vector to the gut of the animal, wherein the
6 vector comprises a non-AAV gene of interest ligated into an AAV vector.

7 8 BRIEF DESCRIPTION OF THE DRAWINGS

9 The invention will be better understood by reference to the following detailed
10 description of the invention when considered in combination with the drawings that form
11 part of the specification, wherein:

12 Figure 1 is a graph showing plasma glucose and animal weight following an
13 acute lactose challenge and a lactose-only diet. A. The change in plasma glucose
14 following the ingestion of lactose in overnight fasted rats. Rats were studied 1 week
15 following AAVlac or PBS administration. B. The oral lactose challenge was repeated
16 after 14 days on the lactose diet. C. The weights of rats at baseline, 1 week and 2 weeks
17 following a 14 day lactose and water diet. The diet commenced 1 week following oral
18 AAVlac or PBS treatment.

19 Figure 2. A. The change in plasma glucose following the ingestion of
20 lactose in overnight fasted rats, which were challenged 120 days following a single
21 peroral dose of AAVlac or PBS. B. The weights of rats at baseline, 1 week and
22 2 weeks following a 14 day lactose and water diet. The diet commenced 120 days
23 following oral AAVlac or PBS treatment.

24 25 DESCRIPTION OF SPECIFIC EMBODIMENTS

26 The present invention is quite straightforward: prior to this invention
27 recombinant AAV vectors were well known and were known to be able to transduce a
28 number of cells and tissues, but had not been used or suggested for use in expressing
29 gene products in the gut of animals. The invention therefore comprises administering to
30 the gut of a target animal a recombinant AAV vector containing a gene whose expression
31 is desired (along with the appropriate control elements, if desired or necessary in the
32 normal manner for vectors). No new vectors are required, as previously known AAV

1 vectors have been shown to work well for gut expression. Thus the invention is in part a
2 discovery that no particular adaption of AAV vectors is required for gut expression,
3 which is surprising in view of the strict requirements for AAV reproduction (i.e.,
4 presence of a helper virus) and the normal association of AAV with the lungs and nasal
5 passages.

6 A number of scientific and patent publications describe the state of the art in
7 the AAV vector field. Since no particular adaptations of prior art vectors are required for
8 practice of the present invention, there is no need here to detail at great length the already
9 well-known state of the art. However, the following publications are herein incorporated
10 by reference, as are the patent and the patent applications (and their published
11 equivalents) identified in the Introduction section of this specification, as these materials
12 may be useful for those less experienced in the AAV field:

- 13
14 1. Samulski, R.J. et al. (1982)
15 *Proc. Natl. Acad. Sci. USA.* 79:2077-2081
16 "Cloning of Adeno-Associated Virus into pBR322: Rescue of Intact Virus
17 from Recombinant Plasmid in Human Cells"
18
- 19 2. Samulski, R.J. et al. (1983)
20 *Cell* 33:135-143
21 "Rescue of Adeno-Associated Virus from Recombinant Plasmids: Gene
22 Correction within the Terminal Repeats of AAV"
23
- 24 3. Laughlin et al. (1983)
25 *Gene* 23:65-73
26 "Cloning of Infectious Adeno-Associated Virus Genomes in Bacterial
27 Plasmids"
28
- 29 4. Hermanot, P.L. and Muzycka, N. (1984)
30 *Proc. Natl. Acad. Sci. USA.* 81:6466-6470
31 "Use of Adeno-Associated Virus as a Mammalian DNA Cloning Vector:
32 Transduction of Neomycin Resistance into Mammalian Tissue Culture Cells"

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1 5. Senepathy, P. et al. (1984)
2 *J. Mol. Biol.* 178, 179:1-20
3 "Replication of Adeno-Associated Virus DNA Complementation of Naturally
4 Occurring rep⁻ Mutants by a Wild-type Genome or an ori⁻ Mutant and
5 Correction of Terminal Palindrome Deletions"
6
7 6. Tratschin et al. (1984)
8 *J. Virol* 51:611-619
9 "Genetic Analysis of Adeno-Associated Virus: Properties of Deletion Mutants
10 Constructed *In Vitro* and Evidence for an Adeno-Associated Virus Replication
11 Function"
12
13 7. Tratschin et al. (1984)
14 *Mol. Cell. Biol.* 4:2072-2081
15 "A Human Parvovirus, Adeno-Associated Virus, as a Eukaryotic Vector:
16 Transient Expression and Encapsidation of the Prokaryotic Gene for
17 Chloramphenicol Acetyltransferase"
18
19 8. Miller et al. (1986)
20 *Somatic Cell and Molecular Genetics* 12:175-183
21 "Factors Involved in Production of Helper Virus-Free Retrovirus Vectors"
22
23 9. Bosselman et al. (1987)
24 *Mol. Cell. Biol.* 7:1797-1806
25 "Replication-Defective Chimeric Helper Provirus and Factors Affecting
26 Generation of Competent Virus: Expression of Moloney Murine Leukemia
27 Virus Structural Genes via the Metallothionein Promoter"
28
29 10. Ohi et al. (1988)
30 *J. Cell. Biol.* 107:304A
31 "Construction and Characterization of Recombinant Adeno-Associated Virus
32 Genome Containing β -globin cDNA"

- 1 "Construction and replication of an adeno-associated virus expression vector
2 that contains human beta-globin cDNA - plasmid PAVh-beta-GHP11 and
3 plasmid PAVh-beta-G-psi-1 construction; potential application in gene therapy
4 of e.g. sickle cell anemia or thalassemia"
- 5
6 17. Ohi, S. et al. (1990)
7 *FASEB J.* 4:7, A2288)
8 "Production and expression of recombinant adeno-associated viruses
9 harboring human beta-globin cDNA - adeno-associated virus expression in
10 293 cell culture; potential gene therapy for hemoglobinopathy disease"
- 11
12 18. Samulski et al. (1991)
13 *Embo J.* 10:3941-3950
14 "Targeted Integration of Adeno-associated virus AAV Into human
15 chromosome 19"
- 16
17 19. Ruffing et al. (Dec. 1992)
18 *J. Virol.* 66:6922-6930
19 "Assembly of Viruslike Particles by Recombinant Structural Proteins of
20 Adeno-Associated Virus Type 2 in Insect Cells"
- 21
22 20. Sitaric et al, (1991)
23 *FASEB* 5:A1550
24 "Production of a Helper-free Recombinant Adeno-Associated Virus That
25 Harbors Human β -globin cDNA"
- 26
27 21. Walsh et al. (1991)
28 *Clin. Res.* 2:325
29 "Gene Transfer and High-level Expression of a human γ -globin Gene
30 Mediated by a Novel Adeno-Associated Virus Promoter"
- 31
32 22. Carter, B.J. (October 1992)

- 1 *Curr. Opinion in Biotechnol.* 3:533-539
2 "Adeno-Associated Virus Vectors"
3
4 23. Ohi et al. (1992)
5 *(June 21-22, 1991) EXP Hematol* 20 119
6 "Synthesis of a human beta globin in the recombinant adeno-associated virus-
7 infected cells towards gene therapy of hemoglobinopathies"
8
9 24. Flotte et al. (1993)
10 *J.B.C.* 268:3781-3790
11 "Expression of the Cystic Fibrosis Transmembrane Conductance Regulator
12 from a Novel Adeno-Associated Virus Promoter"
13
14 25. Wong et al. (1993)
15 *Blood* 82:302A.
16 "High efficiency gene transfer into growth arrested cells utilizing an adeno-
17 associated virus (AAV)-based vector"
18
19 26. Shaughnessey, et al. (1994)
20 *Proc. Am. Assoc. Cancer Res.* 35:373
21 "Adeno-associated virus vectors for MDR1 gene therapy - multidrug-
22 resistance gene cloning and gene transfer into hematopoietic stem cell culture
23 using adeno-associated virus vector CWRSP for potential gene therapy"
24
25 27. Tenenbaum, L. et al. (1994)
26 *Gene Ther.* (1, Suppl.1,S80)
27 "Adeno-Associated Virus (AAV) as a Vector for Gene Transfer into Glial
28 Cells of the Human Central Nervous System - Potential Gene Therapy"
29
30 28. Friedmann, T. (1994)
31 *Gene Ther.* (1, Suppl.1, S47-S48)

"Gene Therapy for Disorders of the CNS - Parkinson Disease Alzheimer
Disease Therapy by Gene Transfer Using Herpes Simplex Virus, Adeno
Virus and Adeno-Associated Virus Vector"

DE 42 19626 A1

Assignee: Wehling, P.

Filed: 16 June 1992

Publication: 23 DEC 93

"Methods for Introducing Therapeutically Relevant Genes into Cells"

WO 91/18088

Assignee: Nat. Inst. Health-Bethesda

Filed: 17 May 1991 (Priority 23 May 1990)

Inventors: Chatterjee and Wong

Publication: 28 November 1991

"Adeno-Associated Virus (AAV)-based Eukaryotic Vectors"

EP 0 592 836 A1

Assignee: American Cyanamide Co.

Filed: 16 September 93 (priority 17 Sept 92 US 947127)

Publication: 20 April 94

"Human Adeno-Associated Virus Integration Site DNA and use thereof"

WO 93/24641

Assignee: U.S. Dept. Health-Human-Serv.

Filed: 2 June 1993 (Priority 2 June 1992)

Publication: 20 APR 94

"Adeno-Associated Virus with Inverted Terminal Repeat Sequences as
Promoter"

WO 93/09239

Assignee: Res. Corp. Technol.

002240 2265560

1 Filed: 6 NOV 92 (US priority 8 NOV 91)
2 Publication: 13 MAY 93
3 "Adeno-Associated Virus-2 Basal Vectors"

4
5 34. EP 0 488 528 A1
6 Assignee: Appl. Immune Sci.
7 Filed: 29 OCT 91 (US priority 30 OCT 90)
8 Publication: 3 JUNE 92
9 "Recombinant adeno-associated Virus Vectors"

10
11 35. USPN 4,797, 368
12 Assignee: U.S. Dept. Health-Human-Serv.
13 Filed: 15 MAR 85
14 Issued: 10 JAN 89
15 "Adeno-associated Virus as Eukaryotic Expression Vector"

16
17 Two recent review article provide a particularly complete overview of the
18 recent status of gene therapy using AAV virus and include a collection of additional
19 recent scientific publications in this field.

20
21 36. Samulski, R. J.
22 "Adeno-associated Viral Vectors"
23 Chapter 3 in "Viruses in Human Gene Therapy"
24 Chapman & Hall, J.-M. H. Vos., ed.

25
26 37. Samulski, R. J.
27 "Adeno-associated Virus-based Vectors for Human Gene Therapy"
28 Chapter 11 in "Gene Therapy: From Laboratory to the Clinic"
29 World Scientific, K. M. Hui, ed.

30
31 Actual delivery of the viral vector for purposes of the invention is
32 accomplished by using any physical method that will transport the AAV recombinant

1 vector to the gut. In this discussion on administration, "AAV vector" means both a bare
2 recombinant AAV DNA vector or AAV vector DNA packaged into viral capsids. Simply
3 dissolving an AAV vector in phosphate buffered saline has been demonstrated to be
4 sufficient for useful gut expression, and there are no known restrictions on the carriers or
5 other components that can be coadministered with the vector (although compositions that
6 degrade DNA should be avoided in the normal manner with vectors). Pharmaceutical
7 compositions can be prepared as oral tablets, capsules, or ingestible liquids or as
8 suppositories. The vectors can be used with any pharmaceutically acceptable carrier for
9 ease of administration and handling.

10 The AAV vector may be orally administered, for example, with an inert
11 diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell
12 gelatin capsules, or it may be compressed into tablets, or it may be incorporated directly
13 with the food of the diet. For oral therapeutic administration, the AAV vector may be
14 incorporated with excipient and used in the form of ingestible tablets, buccal tablets,
15 troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions
16 and preparations should contain at least 1 ug, preferably 10-1000 μ g of AAV vector
17 DNA, or 5×10^3 to 5×10^6 infectious units AAV vector per kg body weight. The
18 amount of AAV vector in a therapeutically useful composition is that which is sufficient
19 to produce gene expression at a therapeutically useful level. Preferred compositions or
20 preparations according to the present invention are prepared so that an oral dosage unit
21 form contains between about 10 and 1000 μ g of AAV vector DNA or 10^4 to 10^6
22 infectious units AAV vector.

23 The tablets, troches, pills, capsules and the like may also contain the
24 following: a binder such as polyvinylpyrrolidone, gum tragacanth, acacia, sucrose, corn
25 starch or gelatin; an excipient such as calcium phosphate, sodium citrate and calcium
26 carbonate; a disintegrating agent such as corn starch, potato starch, tapioca starch, certain
27 complex silicates, alginic acid and the like; a lubricant such as sodium lauryl sulfate, talc
28 and magnesium stearate; a sweetening agent such as sucrose, lactose or saccharin; or a
29 flavoring agent such as peppermint, oil of wintergreen or cherry flavoring. Solid
30 compositions of a similar type are also employed as fillers in soft and hard-filled gelatin
31 capsules; preferred materials in this connection also include lactose or milk sugar as well
32 as high molecular weight polyethylene glycols. When the dosage unit form is a capsule,

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1 it may contain, in addition to materials of the above type, a liquid carrier. Various other
2 materials may be present as coatings or to otherwise modify the physical form of the
3 dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or
4 both. A syrup or elixir may contain the AAV vector, sucrose as a sweetening agent,
5 methyl and propylparabens as preservatives, a dye, flavoring such as cherry or orange
6 flavor, emulsifying agents and/or suspending agents, as well as such diluents as water,
7 ethanol, propylene glycol, glycerin and various like combinations thereof. Of course, any
8 material used in preparing any dosage unit form should be pharmaceutically pure and
9 substantially non-toxic in the amounts employed. In addition, the AAV vector may be
10 incorporated into sustained-release preparations and formulations.

11 Since AAV has in the past been shown to have a broad host range (for
12 pulmonary expression) and has now been demonstrated to be operable in the gut, there
13 are no known limits on the animals in which gut expression can take place, although
14 expression in animals with evolutionarily developed small and large intestines is
15 preferred, particularly in mammals, birds, fish, and reptiles, especially domesticated
16 mammals and birds such as cattle, sheep, pigs, horses, dogs, cats, chickens, and turkeys.
17 Both human and veterinary uses are particularly preferred.

18 The gene being expressed can be either a DNA segment encoding a protein,
19 with whatever control elements (e.g., promoters, operators, ribosome binding sites) are
20 desired by the user, or a non-coding DNA segment, the transcription of which produces
21 all or part of some RNA-containing molecule or anti-sense molecule that is functional in
22 cells. Since the present invention is directed to a route of delivery and to the vector
23 rather than to the material being delivered, there are no limitations on the foreign DNA
24 (non-AAV DNA) being delivered by the vector. While delivery of genes associated with
25 correction of genetic deficiencies related to gut expression is preferred, expression of
26 genes in the gut has the capability of correcting aberrant gene expression in other
27 locations as a result of transport of expression products throughout the body.

28 We have demonstrated the invention by correcting lactose deficiency in the
29 gut. We used a recombinant adeno-associated virus (AAV) expressing β -galactosidase
30 (AAVlac) and delivered the vector to the proximal intestine using a peroral route.
31 Lactase-deficient rats that received AAVlac were able to metabolize an acute lactose load
32 as demonstrated by a rise in plasma glucose. In contrast, phosphate-buffered saline(PBS)-

1 treated controls demonstrated no effect of lactose on plasma glucose. Furthermore, when
2 animals were placed on a restricted, lactose-only diet, PBS-treated rats continued to lose
3 weight over the entire 2-week test-diet period. In contrast, AAVlac-treated animals had
4 no weight loss during the second week. PCR and RT-PCR and histological analysis
5 confirmed intestinal persistence of viral DNA and expression of the vector-encoded β -
6 galactosidase for the life of the animal (extending to 6 months). Moreover, when animals
7 were re-challenged with a lactose load at 3 months after a single AAVlac or PBS
8 treatment, AAVlac animals retained their ability to metabolize lactose and maintained
9 body weight on a lactose diet. These data indicate that oral delivery of an AAV vector
10 can result in long-lasting phenotypic correction of lactase deficiency.

11 This demonstration system was selected both to prove the principle of the
12 invention and to demonstrate the invention in a therapeutically useful mode. Adult-type
13 hypolactasia is genetically determined by an autosomal recessive gene (Sahi et al. Lancet
14 1973 2:823-828). It is the world's most common genetic disorder, afflicting over 50% of
15 the world's population ranging from 100% in some Southeast Asian populations to less
16 than 5% in some Northern European countries (Flatz Human Genet. 1984 36:306-310).
17 Although the symptoms associated with lactose intolerance are relatively mild and readily
18 controlled by omitting lactose-containing foods, there is some debate as to the potential
19 clinical significance of the dietary restrictions which typically accompany lactose
20 intolerance. Specifically, the reduction in calcium-intake associated with complying with
21 a lactose-free diet may lead to an acceleration in the loss of bone mass in the elderly
22 (Flatz 1987 Advances in Human Genet. 16:1-77 NY Plenum Press); and in adolescents
23 and young adults, it may reduce the bone mineral mass (Mobassaleh et al. Pediatrics
24 75:160-166 1985).

25 We elected to study lactase deficiency in the rat as a model of a
26 gastrointestinal genetic disease. We were particularly interested in determining whether
27 we could obtain phenotypic correction using an orally delivered viral vector. We have
28 previously shown that AAV vectors can result in long-term transgene expression in
29 terminally differentiated cells following *in vivo* administration (Birge et al. NEJM 1967
30 276:445-448). AAV has several features which make it particularly attractive for gene
31 therapy. It is a defective, helper-dependent virus, and the wild-type is non-pathogenic.
32 Vectors can be generated which are completely free of helper virus (Bayless et al. 1975

1 NEJM 292:1156-1159). Furthermore, some recombinant AAV vectors retain just 145
2 base terminal repeats with the entire coding sequences removed. In other AAV vectors,
3 non-AAV DNA is operably linked to a vector comprising a double-D AAV genomic
4 segment consisting of 165 basepairs including an internal terminal repeat with D segments
5 at both ends. These vectors therefore are devoid of all viral genes, minimizing any
6 possibility of recombination and viral gene expression. Moreover, unlike adenovirus,
7 they do not appear to elicit any immune response. Another feature of AAV which makes
8 it potentially suitable for an orally based vector is that of hardiness - AAV is resistant to
9 temperature, pH extremes and solvents (Sandler et al. Am. J. Clin. Nutr. 1985 42:270-
10 274). Furthermore, during an active infection in humans, wild-type AAV is typically
11 found in both respiratory tract and gastrointestinal secretions, the gut is therefore a
12 normal host tissue for the virus.

13 Lactose intolerance is most commonly associated with a reduction in intestinal
14 lactase activity. Lactose digestion is dependent on the enzyme, lactase-phlorizin
15 hydrolase (LPH), a microvillar protein which has both galactosidase activity and glycosyl-
16 N-acylsphingosine glucosylhydrolase activities. However, dietary administration of yeast or
17 bacterial β -galactosidase is sufficient to confer the ability to metabolize lactose (Kaplitt
18 et al. Nature Genet. 1994 8:148-154).

19 Most mammalian species are relatively lactase deficient following weaning,
20 although this developmental change in LPH expression does not appear to be simply a
21 reduction in gene transcription. In both humans and rats, although LPH mRNA declines
22 after weaning, it reappears during adulthood. However, this increase in mRNA is not
23 associated with an increase in translation; and adult enzyme levels within the brush-border
24 remain low. It appears that some LPH protein is expressed, but the enzyme is
25 accumulated within the golgi region and is not transported to the brush-border. Based on
26 this information, we conducted the following examples to illustrate the invention. These
27 examples are not to be considered limiting of the invention unless so specified.

28 29 EXAMPLES

30 We decided to demonstrate increased enterocyte expression of β -galactosidase
31 using a viral vector and to obtain brush-border increases in enzyme activity resulting in
32 phenotypic correction in an animal model. We elected to study the adult rat as a genetic

1 model of adult-onset hypolactasia. However, upon screening adult (> 4-month-old)
2 Sprague-Dawley and Fisher rats, a significant number (at least 70%) had persistent
3 lactase activity as determined by a rise in plasma glucose following an oral lactose
4 challenge test. We therefore selected only those animals that had a flat plasma glucose
5 curve following feeding with lactose.

6 Rats were randomized to receive AAVlac or vehicle. The vector (or PBS
7 vehicle) was delivered in lightly anesthetized, fasting rats using an oro-gastric tube.
8 Animals were allowed to recover and placed on a regular rat chow diet. At various times
9 following AAVlac administration, animals were challenged with lactose and plasma
10 glucose samples measured. Moreover, at one week following AAV and again at 120
11 days, animals were put on a lactose-only diet. Animal weights were monitored, and the
12 lactose challenge was repeated.

13 AAVlac DNA persistence and expression was determined using PCR and RT-
14 PCR, *in situ* RT-PCR and X-gal immunohistochemistry. No β -galactosidase (as
15 determined using X-gal staining) expression was observed within the first 3 hours.
16 However, at 6 hours, clear blue (X-gal positive) cells were seen in a characteristic
17 distribution. Moreover, this expression persisted throughout the animals' lifetime with no
18 loss of expression observed. In contrast to the endogenous enzyme activity in lactase-plus
19 animals which is at the tips of the villi and in the brush-border, the vast majority of
20 expression was within the lamina propria, even at 6 hours following peroral
21 administration. On high-power magnification, however, it appeared that some of the
22 enzyme diffused or was transported to the intestinal brush-border.

23 Administration of AAVlac did not effect the weight gain or behavior of any
24 rats fed on regular rat chow. However, on changing to the lactose-only diet, both
25 AAVlac and PBS-treated rats lost weight. Over the first week, this weight loss was
26 identical in both groups and largely reflected a reduction in food intake and a lack of
27 interest in ingesting the lactose. However, in the second week, both groups ingested the
28 lactose. Of interest, the AAVlac animals had no further weight loss; whereas, the PBS-
29 treated animals continued to lose weight at the same rate as over the first week.
30 Moreover, following a lactose challenge, the AAVlac animals had a significant elevation
31 in plasma glucose; whereas, the plasma glucose level of the PBS-treated animals remained
32 flat (Fig. 1).

1 A group of animals was followed for 4 months following a single oral
2 administration of the vector. At 120 days these rats were rechallenged with a lactose load
3 and then recommenced on a lactose-only diet. In a manner similar to the challenge
4 during the first week following AAVlac administration, the vector-treated animals
5 increased plasma glucose whereas the controls had no response. Furthermore, the PBS-
6 treated animals had persistent weight loss on the lactose diet, whereas the AAVlac-treated
7 animals were able to maintain body weight during the second week (Fig. 2).

8 9 Experimental Details

10 Adult (age > 4 months), male Fisher 344 rats were screened using an oral
11 lactose challenge. Rats were fasted overnight. On the morning of the test, a baseline,
12 fasting plasma glucose level was taken from blood obtained from the tail vein. The
13 animals were then administered 2 gr lactose and the plasma glucose was again measured
14 in a tail vein sample at 30 minutes. Plasma glucose was measured using a Beckman
15 Glucose Analyzer II as previously described (During MJ et al. J Clin Invest 1995;
16 95:2403-2408). Rats which had an increase in plasma glucose of greater than 5 mg/dl
17 were excluded from further study. Rats with flat ($\Delta < 5$ mg/dl) were randomized to 2
18 groups: A) AAVlac and B) PBS. Rats randomized to AAVlac were lightly anesthetized
19 with ketamine/xylazine (8/80 mg/kg i.p.) and an oro-gastric tube inserted. 10 microliters
20 of AAVlac (titer 5×10^6 /ml in a carrier solution or 0.5ml PBS or PBS alone was infused.
21 Rats were allowed to recover and returned to *ad libitum* access to water and rat chow.
22 AAVlac is the recombinant AAV vector prepared from pAB11 as described (Goodman
23 et al. Blood 1994 84:1492-1500).

24 Rats were fasted overnight, and blood was taken by nicking the tail vein.
25 Rats were then given a 30 minute access to 2 gram of lactose (Sigma, St. Louis) in their
26 home cages. Thirty minutes from the midpoint of the lactose meal, a second tail vein
27 sample was taken. The blood was immediately centrifuged upon collection and the
28 plasma analyzed for glucose using the Beckman glucose analyzer. In preliminary studies
29 we had determined that a forced oral dose of lactose using either an orogastric or other
30 forced feeding resulted in a highly variable stress hyperglycemic response. Moreover,
31 eating behavior *per se* was insufficient to raise plasma glucose.

1 Rat chow was removed from housing cages and was replaced with 100%
2 lactose (Sigma, St. Louis). *Ad libitum* water access was continued at all times. Animals
3 were weighed at the beginning and at 7 days and 14 days after commencement of the
4 lactose diet. At the end of 14 days the animals, the lactose was removed and the rats
5 were fed regular rat chow.

6 These studies demonstrate the feasibility of administering an AAV vector
7 orally to obtain long-term gene expression. Moreover, there was no loss of expression
8 evidenced over a 6-month period, and phenotypic correction extended to at least 4
9 months.

10 In normal rats, LPH expression is observed within the enterocytes with the
11 protein transported to the brush-border. The expression of β -galactosidase in our study
12 was somewhat atypical in that expression was most apparent in the lamina propria, with
13 little expression in enterocytes. As turnover of enterocytes occurs every 3-5 days, it
14 might be expected that after 4 or 5 days expression would not be seen within this
15 population of cells. Alternatively, a few progenitor cells in the crypts may have been
16 transduced, although we did not observe any persistent gene expression in enterocytes at
17 both the tips of the villi or in the depths of the crypts. In contrast, as early as 6 hours
18 following AAVlac administration, expression was observed within the lamina propria.
19 This finding is consistent with the function of M cells within the gut. M cells are
20 specialized gut epithelial cells which are scattered throughout the intestine but are found
21 most concentrated overlying Peyer's patches and clusters of immune cells. M cells
22 essentially scavenge foreign proteins, viruses and bacteria and rapidly (within 3 hours)
23 transport these foreign agents to the immune cells within the lamina propria. The early
24 expression of vector encoded β -galactosidase within the lamina propria is consistent with
25 this pathway. In high powered sections we were able to see enzyme activity (as
26 demonstrated by X-gal staining) extending down through the enterocytes to the brush
27 border, thus contributing to the phenotypic correction we observed in this model.
28 However, the greatest expression was within the lamina propria. The gut antigen
29 presenting cells (APC) may be the best cells to generate systemic immune responses and
30 are a target for vaccine development (Berns et al. Adv. Virus Res. 1979 25:407-409).
31 Oral AAV vectors may therefore be very attractive choice for immunization. The
32 persistent expression within the vascular lamina propria also suggest this route may be

1 applicable for protein replacement, particularly where release into the portal circulation is
2 desired. For example, this approach may be useful for restoration of portal insulin
3 release in diabetes mellitus. The stable expression of a transgene within the
4 gastrointestinal immune system may also be useful to generate immune tolerance akin to
5 oral antigen approaches (Scrimshaw et al. Am. J. Clin. Nutr. 1988 48:1129-1136).

6 In summary, we have demonstrated that a single peroral administration of an
7 AAV vector can result in persistent expression and long-lasting phenotypic correction.
8 Our data indicates that a "gene in a tablet" strategy using AAV vectors will be useful for
9 a broad range of conditions. Moreover, the lack of toxicity and non-invasiveness of this
10 approach will render oral AAV vectors as a palatable choice compared to current
11 pharmacological treatments.

12 All publications and patent applications mentioned in this specification are
13 herein incorporated by reference to the same extent as if each individual publication or
14 patent application was specifically and individually indicated to be incorporated by
15 reference.

16 The invention now being fully described, it will be apparent to one of
17 ordinary skill in the art that many changes and modifications can be made thereto without
18 departing from the spirit or scope of the appended claims.

1 WHAT IS CLAIMED IS:

2
3 1. A method of expressing a gene product in the gut of an animal, which
4 comprises:
5 administering a recombinant AAV vector to the gut of said animal, wherein
6 said vector comprises a non-AAV gene of interest ligated into an AAV vector.
7

8 2. The method of Claim 1, wherein said vector is administered dissolved or
9 suspended in a liquid pharmaceutically acceptable carrier.
10

11 3. The method of Claim 2, wherein said liquid carrier comprises an aqueous
12 solution.
13

14 4. The method of Claim 1, wherein said vector is administered in a solid
15 pharmaceutically carrier.
16

17 5. The method of Claim 1, wherein said gene comprises a DNA segment
18 encoding a protein operably linked to a promoter operable in said gut.
19

20 6. The method of Claim 1, wherein said administering is by oral ingestion.
21

22 7. The method of Claim 1, wherein said administering is by suppository.
23

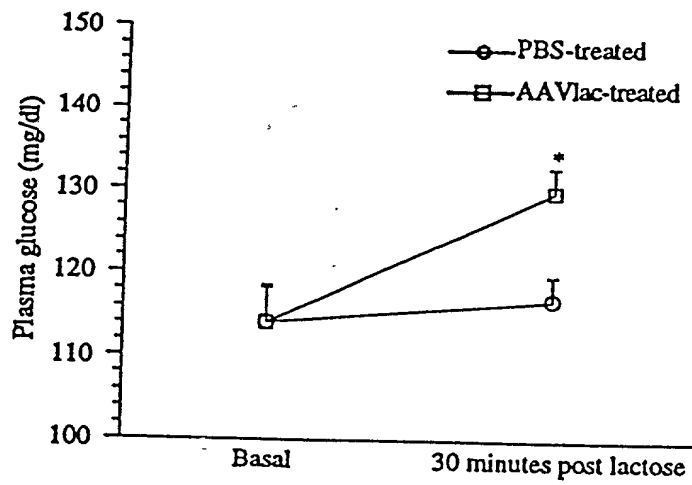
24 8. The method of Claim 1, wherein said AAV vector comprises non-AAV DNA
25 ligated into an AAV genome in place of or in addition to an AAV DNA sequence
26 excluding the first and last 145 basepairs of said AAV genome or non-AAV DNA
27 operably linked to a vector comprising a double-D AAV genomic segment consisting of
28 165 basepairs including an internal terminal repeat with D segments at both ends.
29

30 9. The method of Claim 1, wherein said gene comprises a DNA segment
31 encoding an anti-sense RNA molecule or a genetic control element.
32

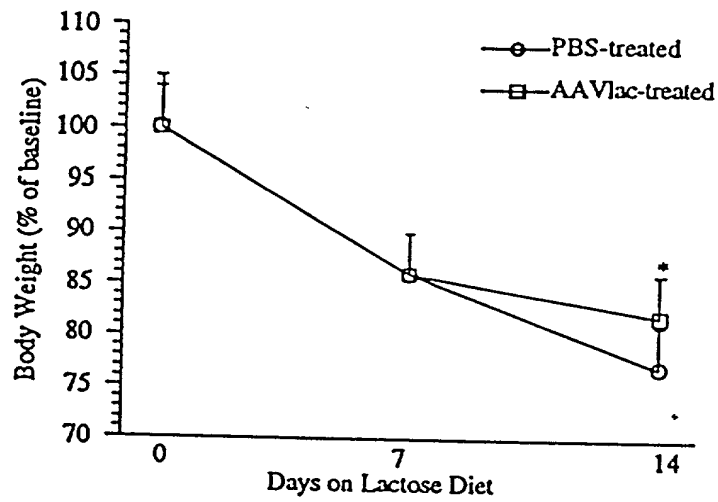
ABSTRACT OF THE DISCLOSURE

A method of expressing a gene product in the gut of an animal, which comprises administering a recombinant AAV vector to the gut of the animal, wherein the vector comprises a non-AAV gene of interest ligated into an AAV vector genome.

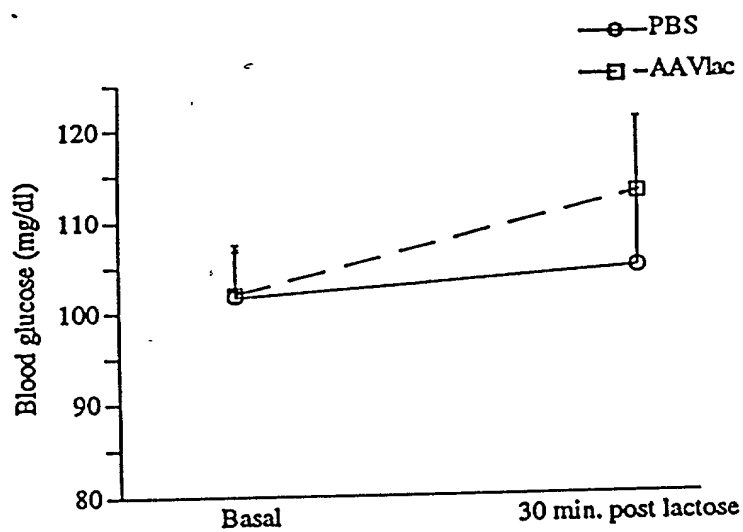
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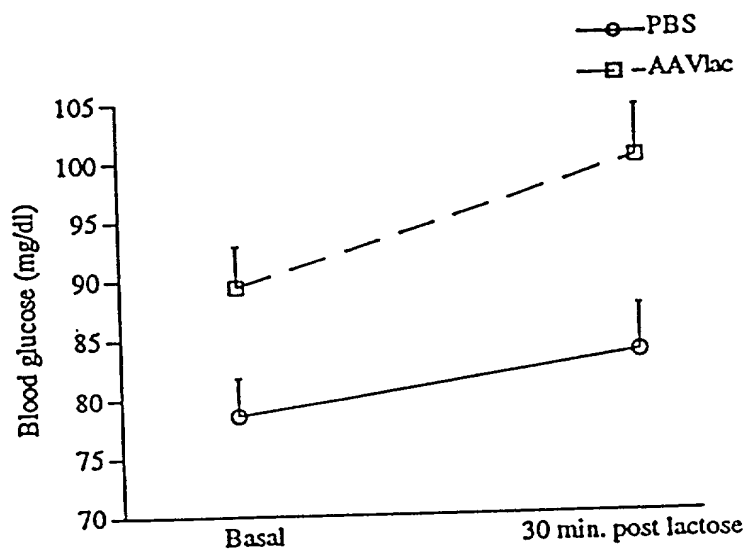
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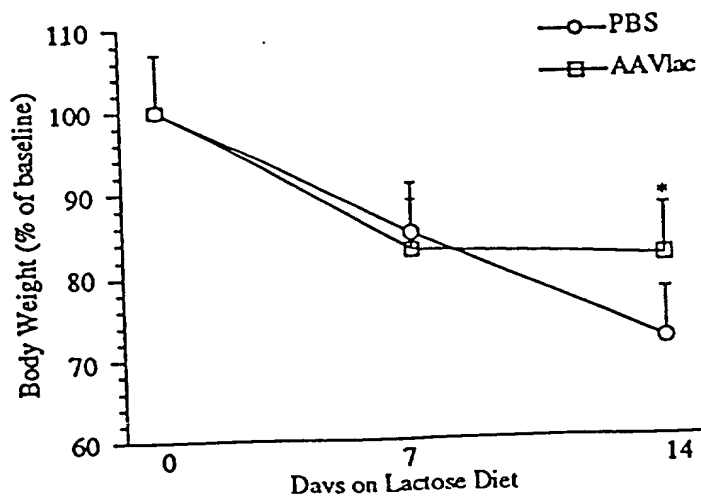
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C



DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ORAL DELIVERY OF ADENO-ASSOCIATED VIRAL VECTORS

the specification of which:

☐ is attached hereto.

☒ was filed on June 7, 1995, and identified as Attorney Docket No. SMTX-006/00US.

☒ was filed on June 7, 1995, as Application Serial No. 08/472,755.

and

☐ the amendment(s) of which were filed on .

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with title 37, code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

<u>Prior Foreign Application(s)</u> (Country) (Number) (Day/Month/Year Filed)	<u>Priority Claimed</u> (Yes/No)
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I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

<u>Appl. Ser. No.</u>	<u>Filing Date</u>	<u>Status (Pat'd./Pend./Aband.)</u>
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I hereby appoint:

John W. Girvin, Jr.	22,706	Marcella Lillis	36,583
Willis E. Higgins	23,025	Craig P. Opperman	37,078
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Luann Cserr	31,822	Kevin J. Zimmer	36,977
Jackie N. Nakamura	35,966		

my attorneys and agents with full power of substitution and revocation to prosecute my above-identified application for Letters Patent and to transact all business in the Patent Office connected therewith.

I further direct that correspondence concerning this application be directed to

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Matthew J. DURING

Appln. No. 08/472,755

Group Art Unit: 1809

Filed: 7 June 1995

Examiner: Nelson

For: ORAL DELIVERY OF ADENO ASSOCIATED VIRAL VECTORS

REVOCATION AND APPOINTMENT OF ATTORNEYS

Assistant Commissioner for Patents
Washington, D.C. 20231

FILED

SEP 18 1997

Sir:

Yale University, owner of the entire right, title and interest in the above-captioned application by virtue of an Assignment from Matthew John During to Yale University recorded on 31 October 1995 at Reel 7708, Frame 0588, hereby revokes all prior powers of attorney and appoints John H. Mion, Reg. No. 18,879; Donald E. Zinn, Reg. No. 19,046; Thomas J. Macpeak, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. 21,092; Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D. Olexy, Reg. No. 24,513; J. Frank Osha, Reg. No. 24,625; Waddell A. Biggart, Reg. No. 24,861; Robert G. McMorrow, Reg. No. 19,093; Louis Gubinsky, Reg. No. 24,835; Neil B. Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 25,916; Joseph J. Ruch, Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland, Reg. No. 32,197;

REVOCATION AND APPOINTMENT OF ATTORNEYS
U.S. Appln. No. 08/472,755

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Respectfully submitted,

Date:

9/17/97

Dorothy K. Roberson
Name:
Title:

40174

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Mathew J. During

Serial No.: Cont. of 08/472,755

Filed: 27 April 2000

For: ORAL DELIVERY OF ADENO-ASSOCIATED
VIRAL VECTORS

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: Group Art Unit: Not Assigned
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: Examiner: Not Assigned
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NOTICE OF CHANGE OF ADDRESS

Assistant Commissioner for Patents
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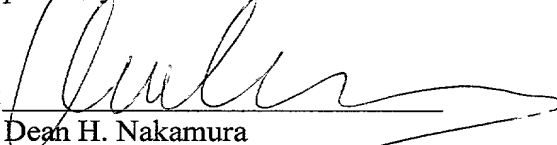
Sir:

The undersigned, attorney for Applicant, hereby gives notice that the mailing address has changed, and requests that the record be amended to show the new mailing address of:

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Please direct all correspondence relating to the instant Application to the undersigned.

Respectfully submitted,

By: 
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Attorney for Applicant
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Dated: 27 April 2000

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